S-2 [10:30～11:00]

Development of a New Herbal Anti-arthritis Drug, Joins™ (SKI 306X)

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Arthritis may be broadly classified as degenerative - related to defects in cartilage and other joint constituents, often age-associated - or inflammatory disease. Inflammatory arthritis called as rheumatoid arthritis (RA) is a chronic inflammatory arthropathy and characterized by a destructive arthritis. RA encompasses infectious arthritis, arthritis caused by intra-articular deposits of crystalline material (gout), syndromes associated with genetic defects (familial Mediterranean fever), and the immune-mediated inflammatory arthropathy.

Degenerative arthritis called as osteoarthritis (OA), which is most frequently occurring, causes degenerative figures of knee, waist and knuckle, and accompanies severe pain around the cartilage. Also, it may cause morning stiffness, gelling effect, tenderness, bone swelling, crepitus, and motion disorders.

However, since there are too many varieties in causes and symptoms, it is generally hard to conclude that arthritis is caused by one or two specific factors.

Up to date, various medicinal herbs have been used for the treatment of paralyzed symptom (痺症, pain, dull sense or insensibility of muscular joint) caused by wind (風), cold (寒), and/or humidity (濕) in East Asia including Korea, China, and Japan.

The paralyzed symptom (痺症) is classified into wind pain (風痺), cold pain (寒痺), and humidity pain (濕熱痺) according to records of oriental traditional medicinal books. In these books, wind pain is a symptom that pain is not localized in a part and shifts all around the body. Cold pain has such a symptom that pain is very severe and is localized in one part of the body. On the other hand, the symptoms of humidity pain are such that pain is fixed on a part and accompanying flare, tumefaction, and the feeling that joint is burning severely. For the treatments of these diseases, anti-inflammatory & detoxificating drug (淸熱藥), sweating & refreshing drug (解表藥), and anti-cold & wind that means anti-germinal drug (去風濕藥) have been used, which could correspond to anti-inflammatory, analgesic, circulation motivating, and antipyretic drug of modern chemotherapies, respectively.

To develop a new herbal anti-arthritis drug, SK Chemicals and SK Pharma scientists selected about sixty medicinal herbs by referencing chinese traditional medicinal books and scientific journals that report various pharmacological activities. On the based of investigation into their anti-inflammatory activity, analgesic effect, blood-circulating improvement, and inhibition activities of articular cartilage break-down enzyme, eventually three medicinal herbs, *Clematis mandshurica*, *Trichosanthes kirilowii*, and *Prunella vulgaris*, were determined as final materials for a new herbal anti-arthritis drug. Later on, Joins™ (SKI 306X) was developed by investigating optimal mixing ratio of the three herbs (1:2:1, w/w), extraction and partitioned active *n*-butyl alcohol fraction. It was demonstrated that Joins™ have multifunction such as anti-inflammation, immunomodulation, the activation of blood microcirculation (Table 1, Table 2). Joins™, also, has effects on Randall-Selitto's test and adjuvant induced arthritis test (Fig. 1). In addition, we demonstrated the protective effects of Joins™ *in vitro* and *in vivo* models. *In vitro*, Joins™ inhibited the rhIL-1α induced proteoglycan degradation in rabbit cartilage explants culture in a concentration-dependent manner. While diclofenac showed some, though not significant, inhibition effects, rofecoxib and dexamethasone revealed none (Fig. 2). *In vivo* study, intra-articular collagenase-injection induced OA-like histological changes were reduced by orally injection of Joins™ (Table 3, Fig. 3).

Because a long-term medicinal treatment is needed to regulate chronic disease efficiently, we performed toxicity studies and verified that Joins™ has no noticeable toxicity (LD_{50} > 5.0 g/kg, daily maximum tolerance dose > 3.0 g/kg/day at 4-weeks study). Based on the results from this pre-clinical study, we obtained a provisional registration approval on the condition of clinical trials from KFDA. A double-blind placebo controlled phase II clinical trial was performed for the determination of optimal dosage and evaluation of clinical efficacy. From this study, it was confirmed that administration of Joins™ by 200 mg *t.i.d.*, was optimal, and also demonstrated that Joins™ is a rather safe drug with high efficacy (Table 4, Fig. 4).

To compare the efficacy and safety profiles of Joins™ with those of diclofenac in sustained release formulation, which is the most widely used NSAIDs in the world, we performed randomized, double-blind phase III clinical trial at multiple centers including Seoul National University Hospital. From this study, Joins™ demonstrated that its efficacy was clinically comparable to that of diclofenac (Table 5), and with respect to safety profile such as the incidence rate of drug-related adverse events, Joins™ was reported to be about 2 times more tolerable than diclofenac (p=0.017) (Table 6). In case of gastro-intestinal adverse event, known as a typical adverse event of the NSAIDs, Joins™ showed 3 times more tolerable than

diclofenac (p=0.021).

From all of these studies, we would like to conclude that Joins™ is an efficient agent for the arthritis, showing excellent profiles of the safety and efficacy with not only the anti-inflammatory and analgesic activities, but also the biologically effective multi-functions including protecting activities for articular cartilage.

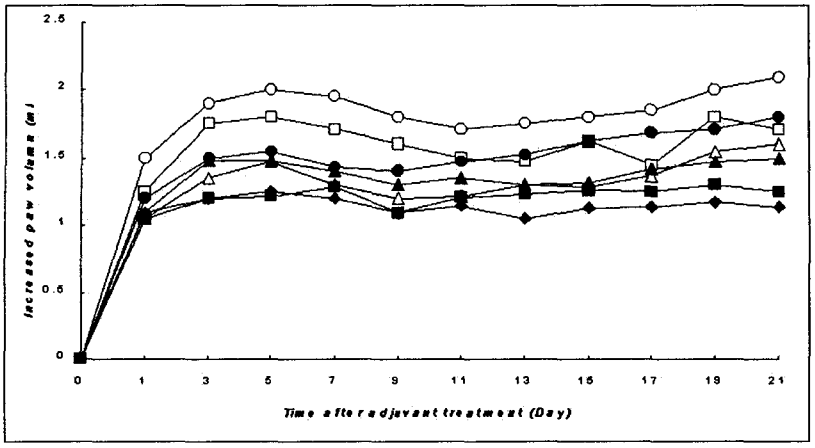
Table 1. Anti-inflammatory effects of Joins™ on ear edema in mice.

Drug	Dose (mg/kg p.o.)	N a)	Thickness increased (mm)			
			croton oil		arachidonic acid	
Control	-	8	0.29 ± 0.02		0.23 ± 0.02	
Aspirin	200	8	0.23 ± 0.04**	(21)	0.19 ± 0.03**	(17)
Joins™	50	8	0.25 ± 0.03**	(14)	0.20 ± 0.02**	(13)
	100	8	0.25 ± 0.02**	(14)	0.18 ± 0.03**	(22)
	200	8	0.23 ± 0.02**	(21)	0.15 ± 0.03**	(35)
	400	8	0.21 ± 0.01**	(28)	0.16 ± 0.02**	(30)
	800	8	0.21 ± 0.03**	(28)	0.13 ± 0.03**	(43)

Each drug was orally administered at 1h before the topical application of 2.5% croton oil or 8% arachidonic acid dissolved in acetone. Each value represents mean ± S.E.,
a) : Number of animals, * ; $p < 0.05$, ** ; $p < 0.01$: significantly different from control,
( ) : Inhibition rate

Table 2. Multifunction effects of Joins™

Anti-inflammation	Relieve Pain & Inhibit chronic inflammation process
Immune -modulation	Inhibit inflammation by immune response (eg. RA)
Blood circulation	Prevent ischemic stress of joint
Cartilage protection	Prevent cartilage degradation



Each point represents the mean. of 8 determinations. Control (○), aspirin 200mg/kg (△), Joins™ 50mg/kg (□), Joins™ 100mg/kg (●), Joins™ 200mg/kg (▲), Joins™ 400mg/kg (■), Joins™ 800mg/kg (◆). * ; $p < 0.05$, ** ; $p < 0.01$: significantly different from control group

Fig. 1. Anti-inflammatroy effect on adjuvant induced arthritis in rats

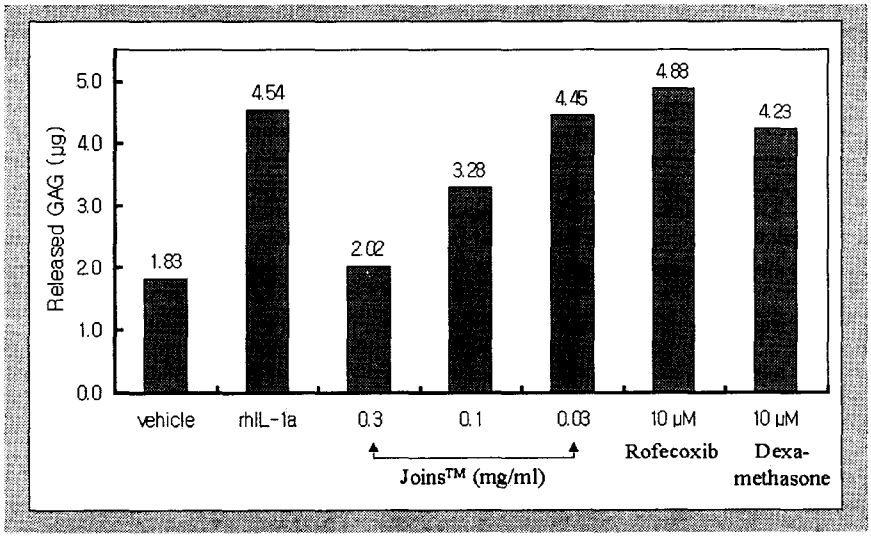


Fig. 2. Inhibitory effect of Joins™ on proteoglycan degradation induced by rhIL-1α.

Table 3. Histological evaluation of articular cartilage and synovial tissue of rabbits (*in vivo* model test)

Treatment group	0.5% CMC (n=5)	diclofenac 10 mg/kg (n=5)	Joins™ 200 mg/kg (n=3)
Articular cartilage Loss of superficial layer Erosion of cartilage Fibrillation and/or fissures Disorganization of chondrocytes Loss of chondrocytes Cluster formation	19.5 ± 2.7	19.3 ± 2.7	12.7 ± 3.0*
Synovial tissue Hyperplasia of synovial lining cell Hypertrophy of synovial lining layer Infiltration of inflammatory cells Proliferation of granulation tissue Vascularization	14.8 ± 2.7	11.9 ± 2.6	9.9 ± 2.8*

* p=0.05 compared to 0.5% CMC treatment group

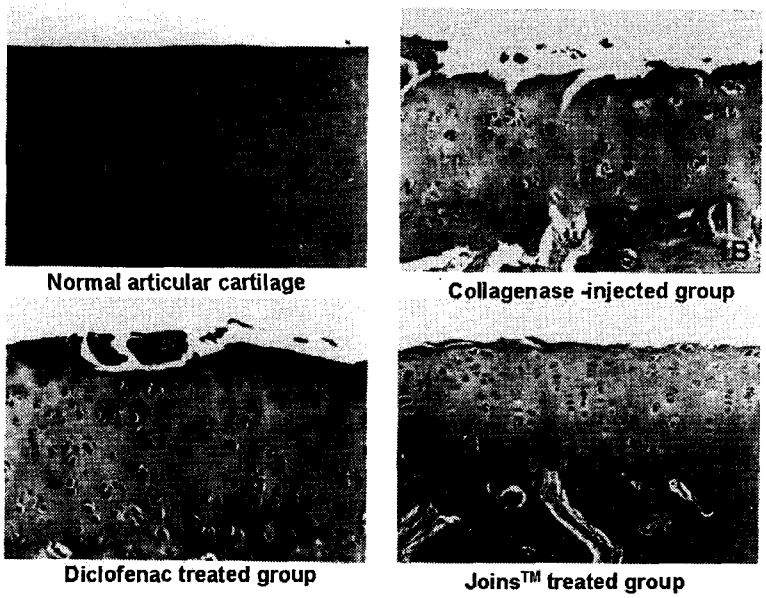


Fig. 3. Representative sections of articular cartilage. (H&E staining. x200.)

Table 4. Results of the efficacy assessments from phase II clinical study of Joins™.

Primary end point, 100mm Visual Analogue Scale

Treatment groups	Placebo group	Joins 200mg group	Joins 400mg group	Joins 600mg group
Mean difference	-7.5±15.5	-23.6±16.3	-22.0±14.0	-29.8±17.4

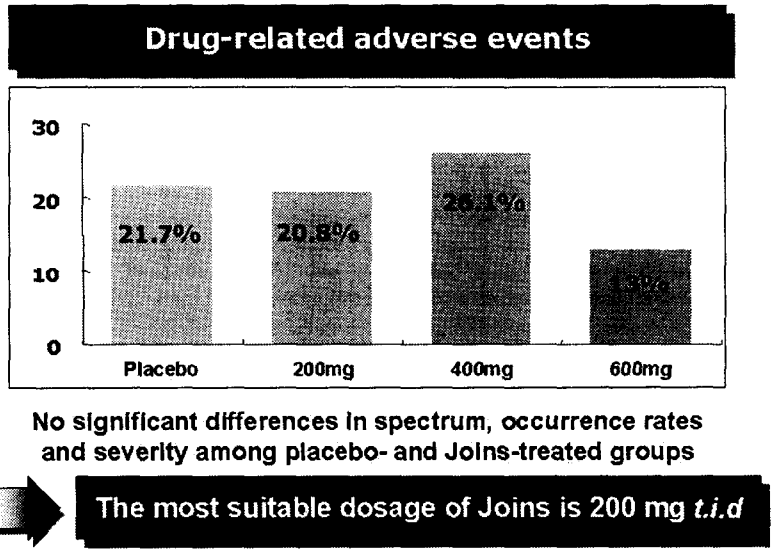


Fig. 4. Results of the safety and tolerability from the phase II clinical study of Joins™.

Table 5. Results of the efficacy assessments from phase III clinical study of Joins™.

100mm Visual Analogue Scale[a]

Treatment group	Joins™ (N=118)	diclofenac (N=119)
VAS	−15.02 ± 17.69	−16.02 ± 15.36

Comparison between Treatment			
Joins™ vs. diclofenac	Difference	95% C.I.	*p*-value
	−0.99	[−4.53, 2.55]	0.64

a: Figures in the table are mean ± S.D.
In the case of halfway-excluded patients,
LOCF(Last Observation Carried Forward) was applied to the data
till the point of exclusion, which were used 4-week data.

Table 6. Results of the safety and tolerability from the phase III clinical study of Joins™.

Drug-related adverse events[*]

Treatment group	Joins™ (N=125)	diclofenac (N=124)
No. of patients showing drug-related adverse events[a]	22 (17.6)	36 (29.0)
Allergy	0 (0.0)	1 (0.8)
Cardiovascular	1 (0.8)	3 (2.4)
Dermatological	1 (0.8)	0 (0.0)
Digestive	19 (15.2)	30 (24.2)
Neurology	0 (0.0)	1 (0.8)
Renal/Genitourinary	1 (0.8)	2 (1.6)
Others	2 (1.6)	9 (7.3)
Total[b]	24 (19.2)	46 (37.1)

* The numbers in the table represent those of adverse events and the numbers in parenthesis represent percentages
a: p=0.033 by Chi-square test, b: p=0.017 by Chi-square test